32 TOXICOLOGY QUALITY GUIDELINES

32.1 Summary

32.1.1 The following toxicology quality guidelines apply for accepting and reporting toxicology results, unless otherwise specified in a specific method SOP. If exceptions are made by the supervisor, document the exception in the case notes.

32.2 Guidelines For Confirming Positive Results

32.2.1 As a general matter of forensic principle, the detection of drugs and other toxins should be confirmed (whenever possible) by a second technique based on a different chemical principle.

32.2.2 If a second technique is not available, the identification must be confirmed on a different extract of the same specimen or in a second specimen.

32.2.3 Whenever possible, the confirmatory (second) test should be more specific and sensitive than the first test for the target analyte. Mass spectrometry is recommended as the confirmatory technique. Exceptions include analytes which are not readily analyzed by mass spectrometry such as carbon monoxide, volatile hydrocarbons (alcohols) and heavy metals.

32.2.4 The following are acceptable confirmatory practices in order of preference. At least one condition must be satisfied in order to identify and report a drug.

32.2.4.1 Identification of the substance class and specific identification of the substance in a different aliquot by a different chemical principle (e.g. immunoassay followed by GC/MS SIM quantitation).

32.2.4.2 Identification of the substance in more than one aliquot by different chemical principles (e.g. base screen identification of antidepressant by GC/MS followed by quantitation by GC/NPD).

32.2.4.3 Identification of the substance in different biological samples by one or more chemical principles (e.g. positive immunoassay for opiates in blood, confirmation of morphine in vitreous humor).

32.2.4.4 Identification of the substance in one biological sample using two separate aliquots and one chemical principle (e.g. ethanol analysis by headspace GC/FID).

32.2.4.5 Identification of an incidental substance by GC/MS in one aliquot and case history verifies the identification (e.g. lidocaine confirmed in base screen by GC/MS in postmortem case involving medical resuscitation).

32.2.5 When mass spectrometry is used in SIM for the identification of an analyte, the use of at least one qualifying ion for each analyte in addition to target ion and retention time match is required. Whenever possible, the use of two analyte qualifying ions is recommended. Acceptance criterion for ion ratios is 20% or 2 SD relative to that of averaged calibrators or controls. However, it is recognized that some ion ratios are concentration dependent and that comparison to a calibrator or control of similar concentration may be necessary.

32.2.6 With LC/MS, ion ratios may be more dependent on concentration and retention time, therefore ratios of plus/minus 30% are acceptable.

32.2.7 Mass spectra with LC/MS and chemical ionization GC/MS are often more simple than electron impact GC/MS. As such, there are often fewer ions available for choices of target and qualifier ions. It may be necessary to run the sample twice, first for quantitation and second for identification with greater ionization energy or fragmentor voltage to produce additional qualifier ions.

32.3 Quality Assurance And Quality Control

32.3.1 Qualitative Assays

32.3.1.1 For immunoassay, each assay must be calibrated with each new lot number of reagent. Once calibrated, only one negative and at least one positive control/reference must be run along with each batch of unknowns.

32.3.1.2 Analyze a minimum of one negative and at least one positive control/reference standard along with unknowns in each chromatographic and rapid presumptive screening test.

32.3.2 Quantitative Assays

32.3.2.1 Calibrators: Solutions prepared from a standard reference material or purchased that are used to calibrate an assay. Where possible, the calibrators should be prepared in a matrix similar to that of the specimens.

32.3.2.2 Prepare a minimum of three different calibrators in each quantitative assay. The concentration of the calibrators should be such that they bracket the anticipated concentration of the unknown specimen(s).

32.3.2.3 Prepare a response curve of area (height) of analyte to area (height) of internal standard ratio versus calibrator concentration. Calculate the analyte concentration by interpolation of the linear plot. The response curve and determined unknown specimen concentration(s) may be generated by hand or by instrument software.

32.3.2.4 Calculate the coefficient of determination (r^2) for the curve. For most applications, an r^2 of greater than 0.985 is acceptable; however, there may be circumstances where an r^2 of 0.975 is minimally acceptable (e.g. LCMS analyses).

32.3.2.5 Evaluate the curve by back-calculating calibrator concentrations against the curve. Values of ± 25% from the target calibrator concentration are acceptable.

32.3.3 Internal standards: Internal standards are required for chromatographic quantitative assays. Use an internal standard with similar extraction, derivatization, and chromatographic properties to the analyte(s) of interest. The use of stable isotope internal standards for selected ion monitoring GCMS is encouraged but not required since well-chosen non-deuterated internal standards may give similar performance.

32.3.4 Controls: Controls in quantitative assays may be purchased or prepared in-house. In-house prepared controls should be prepared from a different manufacturer or different lot of standard material than used in calibrators. If this is not possible, controls should be prepared from a different weighing or dilution from the calibrators.

32.3.4.1 Analyze a negative control and at least one positive control with each quantitative procedure.

32.3.4.2 Positive control range is ± 20% from the target concentration or ± 20% from the mean concentration if controls are tracked and a mean has been previously determined (via tabulation spreadsheets maintained in each laboratory and statewide).

32.3.4.3 A positive control's analyte concentration must be between the lowest and highest calibrator (approximately midrange) used to prepare the response curve.

32.3.4.4 The negative control must indicate that the analyte of interest is absent or below the LOD of the assay. For chromatographic procedures, the negative control is used to determine the LOD of the assay (the smallest blood concentration of a drug needed to give a peak height (area) 3 times the background signal from an extracted blank blood sample).

32.3.4.5 There may be circumstances when ± 30% is an acceptable control range (e.g. to report an insignificant analyte as "present less than…" one of the controls can be within 30% of its calculated value).

32.3.5 Linear range: For most chromatographic assays, the Limit of Quantitation (LOQ) and Upper Limit of Linearity (ULOL) may be administratively defined in terms of the concentration of the lowest and highest calibrator used in the calibration response curve. Alternatively, the LOQ and ULOL must be determined experimentally, data maintained in laboratory records and stated in a specific method SOP.

32.3.5.1 LOD (Limit of Detection)

- GC: The lowest calibrator detectable that is at least three times greater than the blank response.
- GC/MS: The lowest calibrator detectable that has acceptable ion ratios.

32.3.5.2 LOQ: The lowest calibrator included in the calibration response curve whose back-calculated concentration satisfies sections 41.3.2.5 and 41.3.4.4.

32.3.5.3 ULOL. The highest calibrator included in the calibration response curve whose back-calculated concentration satisfies section 41.3.2.5.

32.4 Chromatograhic And Mass Spectral Quality Control

32.4.1 Chromatographic quality control. Some postmortem casework may contain multiple drugs or co-eluting decomposition products that may prohibit adherence to some of the following chromatography guidelines. Exceptions should be documented in case notes or on chromatograms.

32.4.1.1 Retention Time: Retention times for both analyte and internal standard must be within ± 2.0% of the retention time obtained from the calibrators. Larger deviations (10%) are acceptable for assays based on LCMS, particularly with procedures in which the mobile phase programming is non-isocratic.

32.4.1.2 Peak Resolution: To the greatest extent possible, chromatographic peaks used for quantitative analyses should be resolved from interfering peaks such that the valley between adjacent peaks are no greater than 10% of the peak height of interest.

32.4.1.3 Peak Width: Measured at the base of the peak, chromatographic peaks of interest should be at least 10% of SIM window width to permit diagnostic review.

32.4.1.4 Peak Symmetry: Peak shape should be reasonably symmetrical and return to at least 10% of peak height.

32.4.2 Mass spectral quality control.

32.4.2.1 Full scan mass spectral identification: No rigid mass spectral probability based match criteria are defined to identify a drug. Flexibility is given to the experienced interpreter because rigid criteria can lead to misidentification as well as under-identification. The experienced interpreter will base identification on a number of factors, such as, retention time, unique ions, ion abundance, and literature references as well as probability based matching scores.

32.4.2.2 Selected ion monitoring (SIM) identification: When SIM is used for identification of an analyte, whether as part of a quantitative procedure or not, the use of at least one qualifying ion for each analyte in addition to target ions and retention match is necessary. The use of two analyte qualifying ions and one target ion is recommended whenever possible (exceptions include THC and THCA).

32.4.2.3 Acceptance criteria for ion ratios is ± 20% or 2SD relative to the average ratio from all calibrators used in the calibration response curve. However, it is recognized that some ion ratios are concentration dependent and that comparison to a calibrator of similar concentration may be necessary rather than the

average ratio for the curve. Document in case notes when ion ratios are compared to a calibrator of similar concentration.

32.4.2.4 GCMS chemical ionization mass spectra are often simpler than GCMS electron impact spectra and therefore fewer ions are available for the choice of qualifier ions. At least one unique qualifier ion in addition to the target ion is encouraged.

32.5 Criteria For Reporting Toxicology Case Results

32.5.1 Drug reporting guidelines: Report drug concentrations in mg/L on the certificate unless otherwise stated in a specific method SOP.

32.5.1.1 All results are truncated (not rounded). For samples analyzed in duplicate, duplicate results must agree within 20% (except ethanol).

32.5.1.2 If multiple dilutions are analyzed, report the least dilute sample that falls within the linear range of the assay.

32.5.1.3 Report drug concentrations greater than or equal to 0.10 mg/L with two significant digits (e.g. 1.2 mg/L), or truncate to one significant digit (e.g. 1 mg/L).

32.5.1.4 Report drug concentrations less than 0.10 mg/L with one significant digit (e.g. 0.05 mg/L).

32.5.1.5 Report toxicologically significant drug concentrations less than 0.10 mg/L (e.g. THC, fentanyl and LSD) with two significant digits (e.g. 0.015 mg/L)

32.5.1.6 Acquire data with one additional significant digit than stated above. For drugs with low concentrations (THC, fentanyl and LSD) it may be advantageous to collect data in µg/L or ng/ml and then convert to mg/L for reporting.

32.5.1.7 Report drug as "Present" if the drug has been confirmed (section 1.4) but quantitative procedures were not performed or available. Drug may also be reported as "Present" if a quantitative procedure was performed but acceptance criteria were not satisfied and reanalysis is not possible or practical.

32.5.1.8 Report drug as "Present, Less than __" if the confirmed drug has had a quantitative procedure performed but the drug concentration was less than the LOQ of the assay and reanalysis is not possible or practical. Report as "Present, Less Than (LOQ concentration)." If a dilution factor was used in the analysis multiply the dilution factor by LOQ to use for the reported LOQ.

32.5.1.9 Report drug as "Present, Greater than __" if the confirmed drug has had a quantitative procedure performed but the drug concentration was greater than the ULOL of the assay and reanalysis is not possible or practical. Report as "Present, Greater Than (ULOL concentration)." If a dilution factor was used in the analysis, multiply the dilution factor by ULOL to use for the reported ULOL.

32.5.1.10 Report as "Not Detected." Absence of the particular drug(s) within the limitation of the test performed.

32.5.1.11 Report as "Not Detected at __." A quantitative analysis was attempted and the drug did not satisfy confirmation criteria or was below the LOD. The reported concentration is the LOD of the assay. This may also be reported as "Not Detected."

32.5.2 Alcohol reporting. Acquire data in % w/v to four places after the decimal point (e.g. 0.0196 gm%). Calculate the average of the duplicates. Calculate 5% of the average and a ± 5% range. Replicates must be within the ± 5% range or within ± 0.004% (w/v), whichever is greater. Reanalyze the sample if it is outside of tolerance. An exception for duplicate tolerance of up to 20% may be made on tissue homogenates if the exception is noted in the case notes by the toxicologist.

32.5.3 Carbon monoxide. Report whole number percentages. See carbon monoxide SOP for LOQ and ULOL reporting limits.

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